

FIPLIQ: an alternative solution for gynecological and oral cytology

FIPLIQ: uma solução alternativa para exames citológicos ginecológicos e bucais

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ABSTRACT

Introduction: Liquid-based solution for cytology has been developed to improve Pap test. Some liquid media are commercially available, however, due to the high cost there are difficulties in implementing it in the public health programs of many countries. **Objectives:** To study the suitability of alternative liquid media for the collection and preservation of samples for cytologic examinations, comparing the results with the conventional Papanicolaou methodology. **Material and methods:** In this study, 127 different compositions of alternative liquid-based solutions were tested with samples from 10 volunteers for oral cytology and 20 samples from volunteers for cervical cytology. Formaldehyde-isopropanol-phosphate (FIPLIQ) was used to preserve cervical samples prepared and analyzed on the same day and 3, 7, and 15 days after collection, compared with Pap smear. Evaluations on quality and adequacy of cell types, microorganisms or their cytopathic effects, reactive, degenerative and dysplastic cell alterations were performed. **Results:** Samples processed with FIPLIQ showed results similar to those of conventional Pap smear when analyzing staining cytoplasm with indistinct cytoplasm borders, chromatin structure, presence or absence of different types of cell and microorganisms, reparative process, preneoplastic, and neoplastic cell changes; the samples were stored for up to 15 days after collection. **Conclusion:** Preliminary results suggest that FIPLIQ is suitable for the preparation and preservation of cytology specimens for up to 15 days.

Key words: cellular biology; vaginal smears; oral mucosa; cervical intraepithelial neoplasia; epithelial and glandular neoplasms; premalignant lesions.

RESUMO

Introdução: A citologia em meio líquido foi desenvolvida para melhorar o teste de Papanicolaou. Alguns meios líquidos são comercialmente disponíveis, no entanto, devido ao alto custo, há dificuldades para sua implementação em programas de saúde pública em muitos países. **Objetivos:** Estudar a adequabilidade de meios líquidos alternativos para a coleta e a preservação de amostras para exames citológicos, comparando os resultados com a metodologia convencional de Papanicolaou. **Material e métodos:** Neste estudo, 127 diferentes composições de soluções alternativas de meios líquidos foram testadas com amostras de 10 voluntários para citologia oral e 20 amostras de voluntárias para citologia cervical. O fosfato de formaldeído-isopropanol (FIPLIQ) foi utilizado para preservar amostras cervicais preparadas e analisadas no mesmo dia e três, sete e 15 dias após coleta, em comparação com a citologia convencional. Avaliações de qualidade e adequação dos tipos celulares, de microrganismos ou seus efeitos citopáticos, de alterações celulares reativas, degenerativas e displásicas foram realizadas. **Resultados:** As amostras processadas com FIPLIQ apresentaram resultados semelhantes aos do teste convencional de Papanicolaou quando analisados coloração e apagamento de bordas citoplasmáticas, estrutura de cromatina, presença ou ausência de diferentes tipos de células e microrganismos, processo reparativo, pré-neoplásico e alterações celulares neoplásicas; as amostras foram conservadas por até 15 dias após a coleta. **Conclusão:** Os resultados preliminares sugerem que o FIPLIQ é adequado para a preparação e a preservação de espécimes citológicos por até 15 dias.

Unitermos: biologia celular; esfregaço vaginal; mucosa bucal; neoplasia intraepitelial cervical; neoplasias epiteliais e glandulares; lesões pré-cancerosas.

RESUMEN

Introducción: La citología en medio líquido fue desarrollada para mejorar la prueba de Papanicolaou. Algunos medios líquidos son comercialmente disponibles; no obstante, debido al costo elevado, hay dificultades para su implementación en programas de salud pública en muchos países. **Objetivos:** Estudiar la adecuación de medios líquidos alternativos para recolecta y la preservación de muestras para exámenes citológicos, comparando los resultados con la metodología convencional de Papanicolaou. **Material y métodos:** En este estudio, 127 diferentes composiciones de soluciones alternativas de medios líquidos fueron testadas con muestras de 10 voluntarios para citología oral y 20 muestras de voluntarias para citología cervical. El fosfato de formaldehído- isopropanol (FIPLIQ) fue usado para preservación de muestras cervicales preparadas y analizadas en el mismo día, y tres, siete y 15 días después de la recolecta, en comparación con la citología convencional. Se hicieron evaluaciones de calidad y adecuación de los tipos celulares, microorganismos o sus efectos citopáticos, cambios celulares reactivos, degenerativos y displásicos. **Resultados:** Las muestras procesadas con FIPLIQ presentaron resultados similares a los de la prueba convencional de Papanicolaou cuando analizados color y bordes citoplasmáticos mal definidos, estructura de cromatina, presencia o ausencia de diferentes tipos de células y microorganismos, proceso reparativo, pre-neoplásico y alteraciones celulares neoplásicas; las muestras se conservaron hasta 15 días después de la recolección. **Conclusión:** Los resultados preliminares sugieren que el FIPLIQ es adecuado para preparación y preservación de especímenes citológicos hasta 15 días.

Palabras clave: biología celular; frotis vaginal; mucosa bucal; neoplasia intraepitelial cervical; neoplasias glandulares y epiteliales; lesiones precancerosas.

INTRODUCTION

Conventional Pap test, widely used in population studies, has limitations concerning sample suitability with regard to transportation, preservation, fixation and representativeness⁽¹⁻³⁾.

Several authors support the use of liquid-based cytology for cervical screening test, pointing out advantages, since they are less invasive, show better cell distribution and representative smears, with fewer false-negative results. It was reported an improvement in the screening of patients at high risk when using molecular assays and liquid-based cytology⁽⁴⁻⁸⁾. However, the high cost of liquid-based cytology is a limiting factor for its large scale use in population screening for cervical cancer⁽⁹⁻¹¹⁾. Therefore, the development of suitable and accessible alternatives which improves the conventional Pap smear remains relevant.

OBJECTIVES

The aim of this study was to develop a suitable solution for the preservation of samples for cytological examinations.

MATERIAL AND METHODS

Phase I

Oral samples were collected from 10 volunteers from both genders, aged between 20 and 30 years, non-smoking and

non-drinking. The oral mucosa was exfoliated using a conical cytology brush (Kolplast[®], São Paulo, Brazil) and then transferred to a 15 ml plastic Falcon tube (Becton Dickinson[®], New Jersey, USA) containing 2 ml of liquid-based test. This study was approved by the Research Ethics Committee of the Health Sciences Sector of the Universidade Federal do Paraná (UFPR), under opinion no. 777.445 of 20 August, 2014. On the same day and after 3, 5 and 10 days of storage at room temperature, the samples were homogenized for 20 s (AP56 Vortex Phoenix, Labor, São Paulo, Brazil) and centrifuged at 289 × g for 5 min (centrifuge Sigma 4k15). Then, the supernatant was discarded by inversion, the pellet resuspended in 100 µl of test solution and homogenized using vortex. Smears were performed by spreading 50 µl of the pellet on a glass slide, circumferentially with a pipette tip, dried at room temperature and then fixed with absolute ethanol for 30 min, stained by Papanicolaou Staining Method (Newprov[®], Pinhais, Brazil) and mounted with Entellan (Merck[®], Darmstadt, Germany). It was tested 127 different compositions with the following components: ethanol, methanol, acetic acid, isopropanol, formaldehyde, and glutaraldehyde (**Table 1**). Liquid-based with isotonic phosphate buffered saline, pH 7.4 were evaluated. The slides were analyzed by three cytologists, individually. A systematic horizontal mapping was performed using an optical microscope (100×, 400×; Nikon E200), according to a well established cytomorphological criteria⁽¹²⁻¹⁶⁾. After cell preservation and staining of the nucleus and the cytoplasm, the hereinafter called FIPLIQ (formaldehyde 1 ml/dl and isopropanol 1 ml/dl in isotonic phosphate buffered saline, pH 7.4) was selected for further evaluation of cellularity, cellular overlap, presence of microorganisms, and artifacts analysis. The unsatisfactory criteria for discarding liquid-based solutions are: poorly defined chromatin and significant distortion in cell morphology.

TABLE 1 – Reagents and ratios measured in different liquid-based compositions

Reagents	Ethanol	Methanol	Acetic acid	Isopropanol	Formaldehyde	Glutaraldehyde
	99.5	1	0.3	80	1	1
Concentration measured (ml/dl)	97			70	0.5	0.5
	95			50	3	
	70			22		
	77			20		
	50			3		
	30			2		
	20			1		
	10					

Phase II

Cervical samples of twenty women aged 19-64 years were used for the evaluation of the selected solution. For conventional cytology (control group), material from the squamocolumnar junction (SCJ) was collected with Ayre’s spatula and endocervical cells were collected with a tapered brush (Kolplast®, São Paulo, Brazil). The material was spread on a microscope slide and immediately fixed in absolute ethanol. Then, the remaining material was transferred to 15 ml plastic Falcon tube (Becton Dickinson®, New Jersey, USA) containing 2 ml of FIPLIQ solution. On the same day, and three, seven, and 15 days after storage at room temperature, the samples were processed in a liquid-based solution as in the previous item. In this step, the cytological analysis included: suitability and quality of samples; types and stages of cell differentiation observed; presence of microorganisms; reactive, reparative, preneoplastic, and neoplastic cellular changes; establishing comparisons among samples processed by conventional and liquid-based methods.

RESULTS

Among 127 different formulations tested with oral cytological material, eight liquid-based formulations which presented the best results of cell conservation were selected (Table 2). The best liquid-based in this phase was called FIPLIQ (formaldehyde-isopropanol-phosphate) and it was tested in phase II on cervix samples. In phase II, the analysis of samples processed with FIPLIQ presented the best qualitative results in comparison to conventional Pap test. Some FIPLIQ samples showed cytolysis and overlapping cells, but this was not sufficient to determine non-compliance of the method. Only in two samples with FIPLIQ obscuration by polymorphonuclear leucocytes were observed, which determined their exclusion (Table 3). The cell types, microorganisms, reparative, degenerative, preneoplastic and neoplastic cell changes observed in samples with FIPLIQ, within 15 days of cell preservation, were similar to those seen with conventional methodology (Table 4; Figures 1-5). There was no statistically significant difference between the tested methodologies (z -test $p = 0.05$).

TABLE 2 – Preliminary tests: evaluation of the cytological quality of oral mucosa smears after three days of preservation

Composition liquid-based	Nucleus stain	Cytoplasm stain	Cell preservation	Cellular overlap	Artifacts
Formaldehyde 1 ml/dl; isopropanol 1 ml/dl; PBS	SA	SA	SA	UN	SA
Ethanol 20 ml/dl; formaldehyde 1 ml/dl; isopropanol 1 ml/dl; PBS	SA	SA	SA	UN	SA
Ethanol 20 ml/dl; formaldehyde 3 ml/dl; isopropanol 1 ml/dl; PBS	SA	SA	SA	UN	SA
Ethanol 50 ml/dl; formaldehyde 1 ml/dl; metanol 1 ml/dl; isopropanol 1 ml/dl; PBS	SA	SA	SA	UN	SA
Ethanol 10 ml/dl; formaldehyde 1 ml/dl; methanol 1 ml/dl; isopropanol 1 ml/dl; PBS	SA	SA	SA	UN	SA
Ethanol 50 ml/dl; formaldehyde 1 ml/dl; methanol 1 ml/dl; isopropanol 10 ml/dl; PBS	SA	SA	SA	UN	SA
Formaldehyde 1 ml/dl; isopropanol 20 ml/dl; PBS	SA	SA	SA	UN	SA
FIPLIQ	SA	SA	SA	SA	SA

PBS: phosphate buffered saline, pH 7.4; FIPLIQ: formaldehyde-isopropanol-phosphate; SA: satisfactory sample; UN: unsatisfactory sample.

TABLE 3 – Assessment of the suitability and quality of the samples by the Pap smear and FIPLIQ

Parameters	Pap smear	FIPLIQ 0 days	FIPLIQ 3 days	FIPLIQ 7 days	FIPLIQ 15 days
Cellular shape	20	20	20	20	20
Cell preservation	20	19	18	19	20
Chromatin detail	20	19	19	19	20
Cellularity	20	20	20	20	20
Cytolysis	20	19	17	19	19
Cell overlap	15	15	19	18	20
Obscured by PMN leukocytes	20	18	18	18	18
Obscured by RBC	20	20	20	20	20

Cellular shape – no significant alterations; cell preservation: sufficient cytological characteristics to recognize it; chromatin detail: visible; cellularity: sufficient; cytolysis: no significant occurrence; cell overlap: not excessive; obscured by PMN leukocytes; obscured by RBC: no more than 70% of smear area (n = 20).

FIPLIQ: formaldehyde-isopropanol-phosphate; PMN: polymorphonuclear; RBC: red blood cell.

TABLE 4 – Reactive, reparative, degenerative, preneoplastic and neoplastic alterations in cervical cell samples by Pap smear and FIPLIQ on the same day and after 0, 3, 7 and 15 days of preservation

Alterations	Pap smear	FIPLIQ 0 days	FIPLIQ 3 days	FIPLIQ 7 days	FIPLIQ 15 days
Polychromasia	13	16	15	16	16
Cytoplasmic vacuolization	3	3	3	3	3
Perinuclear halos	16	13	10	14	14
Keratohyalin granules	3	3	3	3	3
Outermost cytoplasm edges	9	6	6	8	6
Koilocytes	2	1	1	2	2
Keratinosis	12	16	11	15	13
Thickening nuclear margin	2	5	5	5	4
Kariorrhesis	2	2	1	1	1
Nuclear enlargement	18	17	17	17	17
Binucleation	10	15	16	15	15
Karyopyknosis*	12	12	12	10	12
Lysed nuclei	2	1	2	3	1
Hyperchromasia	9	6	8	8	8
Increased nucleus/cytoplasm ratio	7	8	8	8	8
Karyomegaly	4	4	4	4	4
Irregular chromatin clumping	4	2	2	2	2

FIPLIQ: formaldehyde-isopropanol-phosphate; *karyopyknosis presence, except in superficial cells.

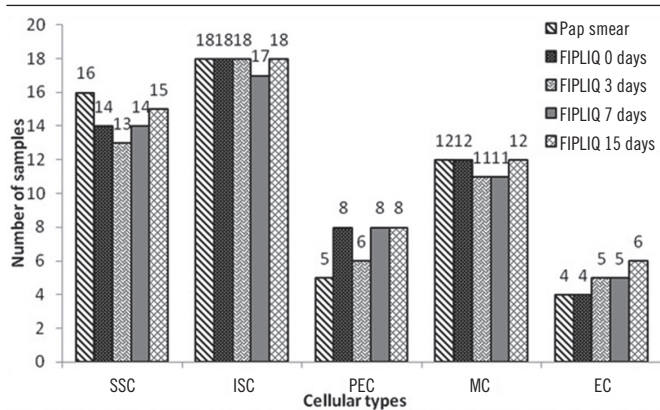


FIGURE 1 – Cell types in cervical specimens from Pap smear and FIPLIQ after 0, 3, 7 and 15 days of cell preservation

There was no statistically significant difference (z-test p = 0.05).

FIPLIQ: formaldehyde-isopropanol-phosphate; SSC: superficial squamous cells; ISC: intermediate squamous cells; PEC: parabasal epithelial cells; MC: metaplastic cells; EC: endocervical cells.

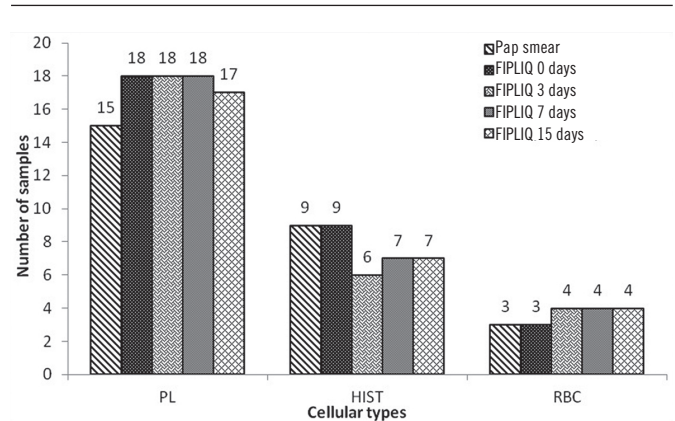


FIGURE 2 – Polymorphonuclear leukocytes, histiocytes and blood in cervical specimens from Pap smear and FIPLIQ after 0, 3, 7 and 15 days of cell preservation

There was no statistically significant difference (z-test p = 0.05).

FIPLIQ: formaldehyde-isopropanol-phosphate; PL: polymorphonuclear leukocytes; HIST: histiocytes; RBC: red blood cells.

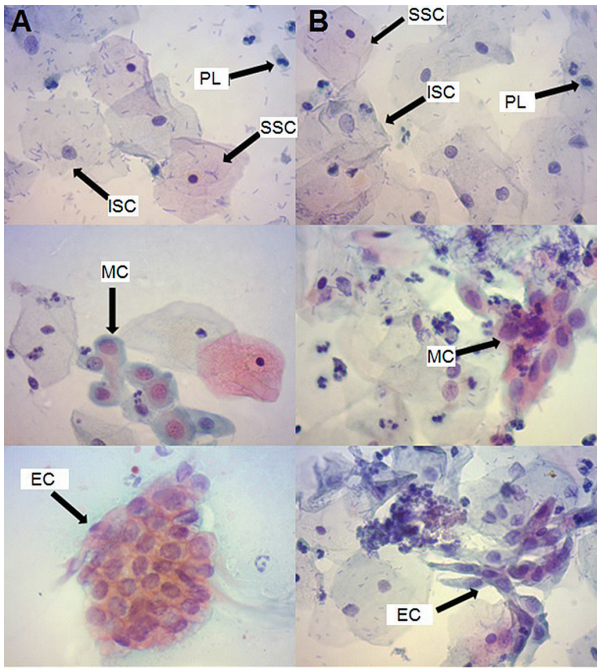


FIGURE 3 – Photomicrographs of cell types observed in cervical specimens by Pap smear (A) and preserved in FIPLIQ after 15 days (B). Papanicolaou stain, 400×

FIPLIQ: formaldehyde-isopropanol-phosphate; PL: polymorphonuclear leukocytes; SSC: superficial squamous cells; ISC: intermediate squamous cells; MC: metaplastic cells; EC: endocervical cells.

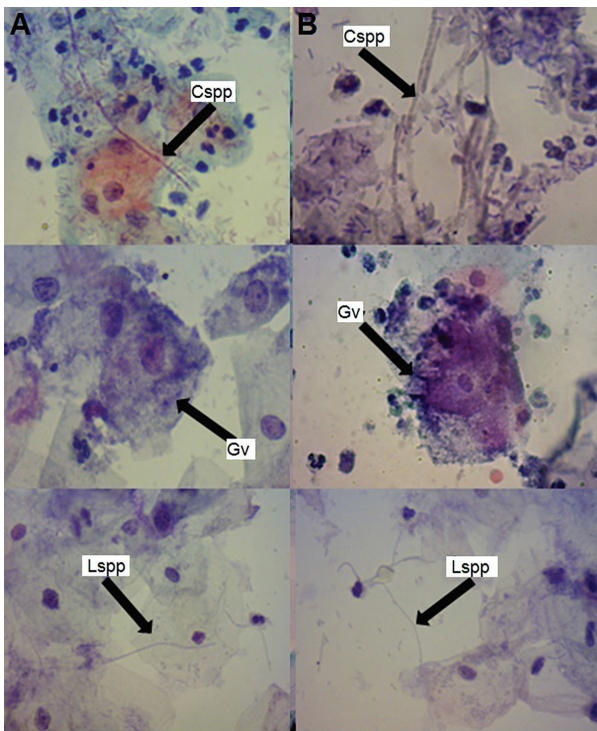


FIGURE 4 – Photomicrographs of microorganisms observed in cervical specimens by Pap smear (A) and preserved in FIPLIQ after 15 days (B). Papanicolaou stain, 400×

FIPLIQ: formaldehyde-isopropanol-phosphate; Cspp: *Candida spp.*; Gv: *Gardnerella vaginalis*; Lspp: *Leptothrix spp.*

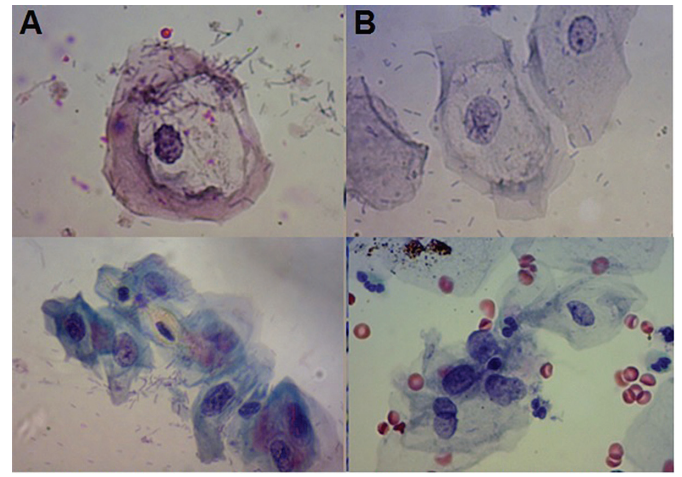


FIGURE 5 – Cytological abnormalities in cervical cells by Pap smear (A) and preserved in FIPLIQ after 15 days (B). Papanicolaou stain, 400×

FIPLIQ: formaldehyde-isopropanol-phosphate.

DISCUSSION

Papanicolaou methodology has been questioned in some studies that point to levels of sensibility of conventional cytological smears with inappropriate representation of cell types. Liquid-based cytology have been described as an improvement of conventional cytology smear as it is related to sample quality, as well as highlighting the possibility of additional tests on the collected material^(1-4, 17-19). Because this method is more expensive than the conventional Pap smear, the implementation of liquid-based cytology in public health systems in developing countries is not feasible^(11, 20, 21). This preliminary study sought to develop more affordable alternatives based on cost. In tests with FIPLIQ, the SCJ remained intact in both methodologies, regardless of time of preservation, proving suitability of collection and morphological conservation of cells. The distinction among the cell types was maintained, allowing their detection (Figures 1-3). The reproducibility among methods that refer to the presence of microorganisms and cytological changes was also studied (Figure 4). Furthermore, the detection rate of epithelial abnormalities in both preparations was similar (Table 2; Figure 5).

When comparing Pap smear and FIPLIQ samples, a similarity in relation to the suitability and quality of the samples was observed (Table 1). Some studies report that liquid-based cytology showed equivalent sensitivity and specificity to conventional Papanicolaou smears and indicate substantial advantage of liquid-based cytology due to the possibility of carrying further tests with the samples^(2, 19, 22).

Other authors, in comparison studies for the performance of oral cytological samples by conventional Pap test and liquid-based, observed that the cell overlap, although reduced, is not eliminated by the liquid-based solution^(22, 23). Standardizing preparation of cytological specimens is difficult, considering the heterogeneity of the samples collected regarding cellularity and other factors, which might not be under the control of the technician, both at the time of collection and processing of the samples. In this work, the cell overlap was an inconvenience in some cases.

CONCLUSION

The results obtained allow us to suggest that FIPLIQ presented similarity in relation to the suitability and quality of the samples when comparing with Pap smear. This method might be used for preservation of cytological of cervicovaginal mucosa samples for 15 days with cell preservation and for preparing quality smears. Further studies may investigate the suitability of the method to other types of cytological specimens.

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